Biologic rhythmic patterns are variable and are affected by disease processes. For instance, heart rate variability (HRV) decreases in severe sepsis. Generally, HRV is measured in the frequency domain, decreases in the 'high-frequency' component of the power spectral density, and reflects an uncoupling of the respiratory influence on heart rate. Cardiorespiratory coupling is mediated by the brainstem, which generates the respiratory pattern. We hypothesized that ventilatory pattern variability (VPV) decreases due to brainstem inflammation prior to the onset of sepsis.

First, we worked to develop methods of measuring VPV that examined properties of the waveform and that distinguished, with caveats, deterministic from stochastic types of variability using iterative Amplitude Adjusted Fourier Transform surrogate data sets that disrupt the nonlinear variability but preserve the linear-dependent properties of VPV. Second, we have initiated *in vivo* experiments to assess the correlation between brainstem inflammation and changes in VPV and cardiorespiratory coupling with developing sepsis. Third, we will apply our analytical tools retrospectively to recorded waveforms from severely ill patient in the Medical Intensive Care Unit at University Hospitals of Cleveland. Third, we will model the brainstem inflammatory response inflammatory response and compare it to the peripheral response and integrate this model with one on the brainstem cardiorespiratory control to predict how inflammation affects control of homeostasis over time.

In this report from the first 6 months of funding, we will focus on the data collection for building the model of the brainstem inflammatory response. Methods: Adult rats (Sprague-Dawley/Harlan, Male, n=15) with sterile (n=2) or *E. coli* (1, 10, 25, 50, 100 (x10^6) bacterial cells) infected clots intraperitoneally. Whole-body plethysmography measured the respiratory pattern of unanesthetized unrestrained rats. The rats were recorded at 18, 20, 22, and 24h post-inoculation. At 24h, the rats were euthanized by anesthetic overdose (Isoflurane). We harvested peripheral and central tissue to measure cytokines: Periphery (Lungs, Broncho-Alveolar Lavage Fluid (BALF), Liver, Heart and Serum and Central Nervous System (Cervical Spinal Cord, Cerebellum, Cerebrum, Pons, Dorsal and Ventral Medulla). Tissues samples were ground in lysing buffer and centrifuged. Supernatants and bodily fluids were analyzed for IL-1β and TNFα using an enzyme-linked immunosorbent assay (ELISA). We measured VPV in the time domain (cycle periodicity and Poincaré Plots) and assessed nonlinear dynamics (Mutual Information and Sample Entropy).

The ventilatory pattern of the infected rats had a greater breathing frequency (f_R) and but minimal changes in its coefficient of variation (CVf_R) compared to the surgical sham rats. Further, measures assessing the distribution of successive cycles in the Poincaré Plots were less in septic versus sham rats. Finally, group data for the values of the nonlinear characteristics of ventilatory waveform indicated that the ventilatory pattern became more predictable. In particular, the nonlinear complexity index (NLCI), measure of the difference between the Sample Entropy of the original and surrogate data sets was greater in the infected than the sham rats. The NLCI reflects the increased deterministic compared to stochastic variability in the waveform.

Preliminarily, IL-1β but not TNFa increased in the brainstem areas generating the respiratory pattern but also in the cerebrum. In particular, brainstem nuclei which generate the ventilatory pattern appear to become inflamed prior to the increases in measure levels in the serum.

In summary, our data are consistent with site-selective effects of cytokines attenuating cardiorespiratory coupling during the inflammatory response.